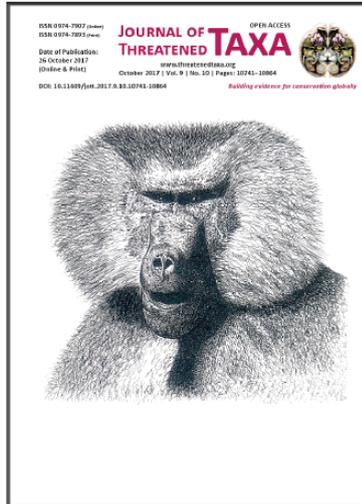


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SHORT COMMUNICATION

A NEW DISTRIBUTION REPORT OF THE CRITICALLY ENDANGERED *AMOMUM KINGII* BAKER (ZINGIBERACEAE) OUTSIDE SIKKIM, INDIA

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A NEW DISTRIBUTION REPORT OF THE CRITICALLY ENDANGERED *AMOMUM KINGII* BAKER (ZINGIBERACEAE) OUTSIDE SIKKIM, INDIA

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Abstract: In this correspondence, the distribution of a population of *Amomum kingii* Baker, a Critically Endangered species of Zingiberaceae, is reported from Darjeeling District, West Bengal, India. Previously, this plant was described only from Sikkim, India and no other population of this endemic plant has ever been found outside this distributional range. In the present study, the plant was morphologically described and molecular characterization was done for the first time using three DNA regions- ITS, *matK* and *rbcl*. The urgency and necessity of conservation of this plant has also been discussed.

Keywords: *Amomum kingii*, Darjeeling, ITS, *matK*, new distribution, *rbcl*.

The genus *Amomum* Roxburgh is the second largest among the 53 genera of Zingiberaceae (Xia et al. 2004). It is represented by about 150 species (Thomas & Sabu 2015) distributed mainly in the tropical and subtropical regions of the world with its centre of diversity in the Indo-Malayan region (Sabu 2006). In India, 28 species of this genus are found (Thomas et al. 2015) mostly in the northeastern and peninsular regions. Of these 28 species, about seven species are considered to be wild and endemic to eastern Himalaya (Partap et al. 2014).

Amomum kingii Baker is one of the endemic species of this genus from eastern Himalaya. It was first described from Sikkim, India by Baker (1892) and was included in the Flora of British India by Sir J.D. Hooker (1894). Later

on, Schumann (1904), Smith (1994) and Kumar (2001) also included this species among Zingiberaceae from eastern India, but none of them could locate any living population of *A. kingii* and they relied on the information provided by Baker (1892). Since the description of Baker (1892), no live specimen of *A. kingii* had been recorded until Thomas & Sabu (2015), while doing a revisionary work on the genus *Amomum* from India, rediscovered a sole population of the species from Pangthang Forest Block in Eastern Sikkim, the same region as reported by Baker. Subsequently, they reported another population of *A. kingii* that was morphologically different from the plant described by Baker (1892) and established it as a new variety—*Amomum kingii* var. *oblongum* (Thomas et al. 2015). Except these, there were no reports of the plant from any other part of the world.

In this correspondence, we report a population of *Amomum kingii* from a locality in West Bengal. The plants were morphologically described and DNA-based molecular characterization of ITS, *rbcl* and *matK* regions were performed for the first time. To the best of our knowledge, this is the only report of *A. kingii* from any region outside Sikkim.

Material and Methods: Plants of *Amomum kingii* were encountered during a field trip in Darjeeling District,

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West Bengal, India in the month of April 2015, and again in 2016. The plants were photographically recorded during both the trips and voucher specimens prepared for submission in the herbarium. Both the photographs and the voucher specimen were authenticated by Prof. Mamiyil Sabu, Department of Botany, Calicut University, Kerala. The voucher specimen was deposited in Calicut University Herbarium, Kerala, India (CALI). Detailed morphological diagnosis was done based on the living materials collected in the field using relevant references (Baker 1892; Sabu 2006; Thomas et al. 2015; Thomas & Sabu 2015).

Young leaves of the studied plants were used to extract their genomic DNA following the protocol of Bhadra & Bandyopadhyay (2015). Three DNA regions were amplified and sequenced using the extracted DNA. The details of the primers used for each gene region and the standardized PCR protocol are given in Table 1. The amplified forward and reverse sequences of each gene region were used to prepare contiguous sequences using the software CAP3 (Huang & Madan 1999). These sequences were then submitted to GenBank.

Results and Discussion: The population of *Amomum kingii* was found in flowering condition in Jhandi dara (27.06666667 N & 88.43333333 E) of Darjeeling District, West Bengal, India in the month of April 2015 and again in 2016. The place is situated about 49km west of the town of Kalimpong in Kalimpong subdivision of Darjeeling District, West Bengal, India, at an elevation of 1,890m, near Neora Valley National Park. The observed population was found to consist about eight plants within about a 2sq.km area, all of which bore inflorescences. The accession number of the voucher specimen submitted in Calicut University Herbarium is CALI 6880.

Taxonomic description of the plant is as follows: Rootstock creeping just below the soil surface. Leafy stem stout, unbranched, up to seven shoots present in each plant. Leaves petiolate, distichous, plane of distichy perpendicular to rhizome, oblong-lanceolate in shape, tip acuminate, base oblique, lamina glabrous, ligulate, ligule dark maroon, up to 11 leaves per shoot. Inflorescence radical, 3–11 inflorescences borne on rhizome near the base of the leafy shoot, compact oblong spike, up to 20cm long, bracteate, bracteolate. Flowers aromatic. Bracts linear lanceolate, membranous, pale yellow, acute, each subtending a single flower. Bracteoles acute. Calyx three-toothed, white. Corolla tube pale yellow, three-lobed, lobes white tinged with pink. Labellum obovate, obscurely 3-lobed, white tinged with yellow, longer than corolla segments, centre with dark

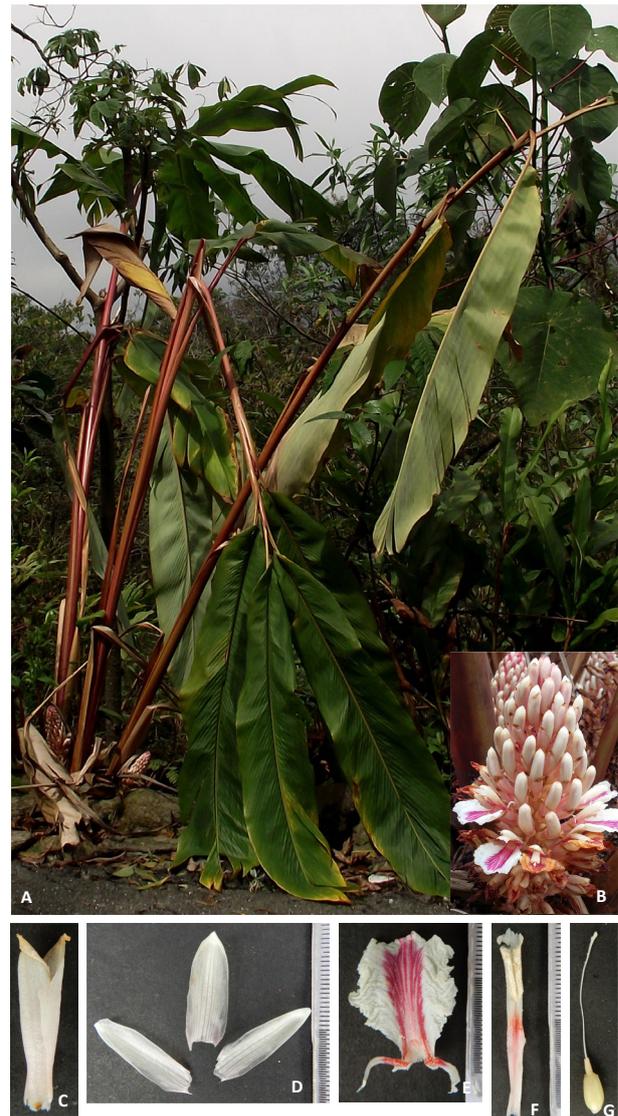


Image 1. *Amomum kingii*: A - Habit; B - Inflorescence; C - Calyx; D - Corolla; E - Labellum; F - Stamen; G - Gynoecium. © Authors

pink bands, margin crenulate. Lateral staminodes flat, pale yellow, lower part maroon. Stamen 1, shorter than labellum. Filament short, creamy white, pink towards base. Anther 2-celled, thecae parallel, oblong, linear. Epigynous glands two, free from each other. Ovary inferior, trilobular with many ovules in axile placentation. Style long, filiform, passing through a groove in the filament. Stigma white, hairy. Fruit globose or spherical capsule, red tinged with green. Seeds reportedly many, though not observed by the authors (Image 1 A–G).

DNA extracted from the young leaves of the plants was subjected to PCR amplification. The sequence of ITS, *matK* and *rbcL* of this plant were submitted to NCBI and were the first ever submissions for the species. The GenBank accession numbers of the sequenced regions

Table 1. Details of the primers used, standardized PCR protocol and GenBank accession number of the submitted sequence

	Amplified DNA regions	Primer type	Primer name	Primer sequence (5'-3')	References of primers used	Tm of primer	PCR condition	GenBank accession number
1	ITS	Forward Primer	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)	51.1°C	95°C for 3 minutes; 25 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 minute; 72°C for 10 minutes	KX774413
		Reverse Primer	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)	49.7°C		
2	matK	Forward Primer	MatK-1RKIM-f	ACCCAGTCCATCTGGAAATCTTGGTIC	Dunning & Savolainen (2010)	59.5°C	94°C for 4 minutes; 25 cycles of 94°C for 1 minute, 46°C for 1 minute, 72°C for 1 minute; 72°C for 10 minutes	KX774414
		Reverse Primer	MatK-3FKIM-r	CGTACAGTACTTTGTGTTTACGIG	Dunning & Savolainen (2010)	54°C		
3	rbcL	Forward Primer	rbcLa_F	ATGTACCACAAAACAGACTAAAGC	Levin et al. (2003)	56.4°C	95°C for 3 minutes; 25 cycles of 95°C for 30 s, 54°C for 30 s, 72°C for 1 minute; 72°C for 10 minutes	KX774412
		Reverse Primer	rbcLa_R	GTAATAATCAAGTCCACCRGC	Kress & Erickson (2007)	49.7°C		

are provided in Table 1.

Transplantation of rhizomes to the Experimental Garden, Department of Botany, University of Calcutta, was attempted but had failed during both the trips. Thomas & Sabu (2015) had earlier reported seed production in the population of *Amomum kingii* studied by them from Sikkim. Bumblebee visitations were documented to *A. kingii* inflorescence during the present study. However, the quest for seeds from all the flowering plants of present population failed, which was also confirmed by the local inhabitants.

Incidentally, all the plants of this population studied were found to be growing along newly constructed metalled roads or were discretely present within *Amomum subulatum* Roxb. plantations. It can be speculated that more plants of the species might have been uprooted during road construction work going on in the region or during clearing of the area for cultivation. It is pertinent to mention here that this region witnessed massive landslides during the rainy season of 2015, so this population too remains vulnerable.

Darjeeling District of the state of West Bengal, where this plant population was found, is in the part of the eastern Himalayan region. While India is one of the 12 megadiversity centers of the world, the eastern Himalaya is a part of the four biodiversity hotspots of the country of the total 35 present across the globe (Williams et al. 2011). Biodiversity hotspots are those regions that have exceptional concentrations of endemic species and experiencing exceptional loss of habitat (Myers et al. 2000). Myer et al. (2000) reported that most of the hotspots (including the eastern Himalaya) are located in the tropical regions of the developing countries where conservation resources are very scarce. Apart

from climatic changes, an alarming rate of population growth, rapid urbanization leading subsequently to deforestation and shifting cultivation pose constant threats to biodiversity in this area (Chitale et al. 2014). According to Jacob et al. (2015), biodiversity loss due to anthropogenic intervention has been more rapid over the last 50 years. Along with population explosion (14% in last decade in Darjeeling District, according to Government of India, 2013) (Sandhu & Sandhu 2015), ecotourism in this area is gaining popularity amongst the common people of the region. Increasing influx of tourists is leading to rapid deforestation and pollution, which is threatening the local flora with the risk of extinction (Chitale et al. 2014).

Unfortunately, *Amomum kingii* falls under the Critically Endangered category of IUCN and attempts at relocation of this species to other areas have reportedly failed (Thomas & Sabu 2015). Uncontrolled anthropogenic activities in the Darjeeling District are potentially contributing in increasing the risk of extinction to the species. Other than attempts of in situ conservation, strategies like micropropagation and ex situ conservation at higher altitudes might be effective and as such these strategies should be employed urgently to save this species from extinction.

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Articles

The relationship between artificial food supply and natural food selection in two troops of commensal *Hamadryas Baboons* *Papio hamadryas* (Mammalia: Primates: Cercopithecidae) in Saudi Arabia

-- Ahmed Boug, M. Zafar-ul Islam, Toshitaka Iwamoto, Akio Mori, Akihiro Yamane & Amy L. Schreier, Pp. 10741–10756

Pollination ecology of *Rhynchosia cana* (Willd.) DC. (Fabaceae), an erect sub-shrub, in peninsular India

-- A.J. Solomon Raju & K. Venkata Ramana, Pp. 10757–10770

Communications

Feeding habits and behaviour of *Bagre bagre* and *Genidens barbatus*, two ariid catfishes (Pisces: Siluriformes) from southeastern Brazil

-- Maria Thereza Manhães Tavares & Ana Paula Madeira Di Benedetto, Pp. 10771–10775

The Odonata (Insecta) of northern and central Oman

-- Elaine M. Cowan & Peter J. Cowan, Pp. 10776–10791

Illustrated description and notes on biology of *Dicranocephalus lateralis* (Signoret) (Coreoidea: Stenocephalidae) from Maharashtra State, India

-- Balasaheb V. Sarode, Nikhil U. Joshi, Swapnil S. Boyane, Subodh S. Gaikwad, Pratik P. Pansare & Hemant V. Ghate, Pp. 10792–10803

Floristic diversity of the Indian Cardamom Research Institute campus, Myladumpara, Western Ghats, India

-- Anoop P. Balan & S. Harikrishnan, Pp. 10804–10822

Short Communications

Mammals of Papikonda Hills, northern Eastern Ghats, India

-- Vikram Aditya & Thyagarajan Ganesh, Pp. 10823–10830

New records of the subfamily Stratiomyinae (Diptera: Stratiomyidae) from Pakistan

-- Muhammad Asghar Hassan, Imran Bodlah, Zafar Iqbal & Rubina Jabeen, Pp. 10831–10834

A new distribution report of the Critically Endangered *Amomum kingii* Baker (Zingiberaceae) outside Sikkim, India

-- Sreetama Bhadra & Maumita Bandyopadhyay, Pp. 10835–10838

A note on the population status and threats on two endemic and Endangered species of *Garcinia* of Agasthyamalai Biosphere Reserve, Tamil Nadu, India

-- G. Manikandan & R. Ramasubbu, Pp. 10839–10845

Feasibility study on the vegetative propagation of four endemic rare balsams (*Impatiens* spp.) through stem cuttings for conservation and management in Idukki District, Kerala, India

-- G. Prasad, P. Rajan & N. Bhavadas, Pp. 10846–10849

Notes

Observation of Shaheen Falcon *Falco peregrinus peregrinator* (Aves: Falconiformes: Falconidae) in the Nilgiris, Tamil Nadu, India

-- Arockianathan Samson, Balasundaram Ramakrishnan, Palanisamy Santhoshkumar & Sivaraj Karthick, Pp. 10850–10852

A first report of *Redigobius oyensi* (de Beaufort, 1913) (Teleostei: Gobionellinae) from Car Nicobar Island, India

-- J. Praveenraj, R. Kiruba-Sankar, Lohith Kumar, J. Raymond Jani Angel & S. Dam Roy, Pp. 10853–10855

New butterfly records from the Jammu Shiwaliks, Jammu & Kashmir, India

-- Shakha Sharma & Neeraj Sharma, Pp. 10856–10859

Range extension of the Indian Tortoiseshell *Aglais caschmirensis aesis* (Fruhstorfer, 1912) (Lepidoptera: Nymphalidae) into the hills of Manipur, India

-- Jatishwor Singh Irungbam, Harmenn Huidrom & Baleshwar Singh Soibam, Pp. 10860–10864